

State of Hawaii  
DEPARTMENT OF LAND AND NATURAL RESOURCES  
Division of Aquatic Resources  
Honolulu, Hawaii 96813

April 24, 2009

Board of Land  
and Natural Resources  
Honolulu, Hawaii

Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National Monument Research Permit to Dr. Ruth Gates, University of Hawaii, Hawaii Institute of Marine Biology, for Access to State Waters to Conduct Coral-Endosymbiont Research Activities.

The Division of Aquatic Resources (DAR) hereby submits a request for your authorization and approval for issuance of a Papahānaumokuākea Marine National Monument research permit to Dr. Ruth Gates, associate researcher professor, University of Hawaii, Hawaii Institute of Marine Biology, pursuant to § 187A-6, Hawaii Revised Statutes (HRS), chapter 13-60.5, Hawaii Administrative Rules (HAR), and all other applicable laws and regulations.

The research permit, as described below, would allow entry and research activities to occur in the Papahānaumokuākea Marine National Monument (Monument), including the NWHI State Marine Refuge and the waters (0-3 nautical miles) surrounding the following sites:

- Nihoa Island,
- Necker Island (Mokumanamana),
- French Frigate Shoals,
- Gardner Pinnacles,
- Maro Reef
- Laysan Island,
- Lisianski Island, Neva Shoal,
- Pearl and Hermes Atoll,
- Kure Atoll State Seabird Sanctuary

The activities covered under this permit will occur from June 1, 2009 through August 31, 2009.

The proposed activities are a renewal of work previously permitted and conducted in the Monument.

INTENDED ACTIVITIES

The purpose of these activities is to identify and monitor biological indicators of coral disease and/or bleaching and assess the diversity of coral-endosymbionts in Papahānaumokuākea.

Specifically, the applicant would characterize the diversity of symbiotic dinoflagellates (Symbiodinium) harbored by corals and examine morphological traits in healthy, diseased and bleached corals in order to identify biological traits that correlate with health state.

To perform these activities, the applicant would sample coral colonies and other marine invertebrates and single-celled protists that harbor the same dinoflagellate symbionts as well as the surrounding reef waters and sediment to determine the molecular diversity of coral endosymbionts. These activities would be performed using SCUBA from small boat operations launched from the NOAA Ship HI'IALAKAI.

A maximum of 30 coral colonies (5 species; 10 healthy, 20 unhealthy) would be sampled from reefs surrounding each atoll. This number is much greater than what the actual "take" number will be, as the occurrence of bleaching and disease in all the listed species at all atolls is extremely rare, and all atolls would not be visited during the cruise. The sample taken from each coral is a very small biopsy (<1cm). The impact of this sampling to is significantly less damaging to the coral than marine organisms that feed on coral in the area (e.g. parrot fish). The sampling impacts are thus extremely minimal and pose no significant threat to the survival of the colony sampled.

A total of 5 colonies per marine invertebrate species (27 species) and 40 Foraminifera per species (3 species) from reefs surrounding each atoll would also be sampled. Marine invertebrates and Foraminifera would be collected with chisel, pliers or scissors and placed into collection bags.

For a detailed sample list, see F-7c.

Samples would be stored in tubes containing DNA preservation buffer that destroys living material. These samples would be transported to HIMB upon return to Honolulu.

The applicant notes that samples that overlap with the Rappe, Toonen, and Karl lab would be shared.

This research would help identifying biological indicators of disease, revealing the diversity of coral-endosymbionts in Papahānaumokuākea, and developing capacity to predict the effects of global climate change on coral reef ecosystems in the Monument.

The activities proposed by the applicant directly support the Monument Management Plan's priority management needs 3.1 – Understanding and Interpreting the NWHI (through action plan 3.1.1 – Marine Conservation Science).

The activities described above may require the following regulated activities to occur in State waters:

- ☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving monument resource
- ☒ Touching coral, living or dead

- ☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

#### REVIEW PROCESS:

The permit application was sent out for review and comment to the following scientific and cultural entities: Hawaii Division of Aquatic Resources, Hawaii Division of Forestry and Wildlife, Papahānaumokuākea Marine National Monument (NOAA/NOS), NOAA Pacific Islands Regional Office (NOAA-PIRO), United States Fish and Wildlife Service Hawaiian and Pacific Islands National Wildlife Refuge Complex Office, and the Office of Hawaiian Affairs (OHA). In addition, the permit application has been posted on the Monument Web site since March 11th, giving the public an opportunity to comment. The application was posted within 40 days of its receipt, in accordance with the Monument's Public Notification Policy.

#### **Comments received from the scientific community are summarized as follows:**

Scientific reviews support the acceptance of this application. No concerns were raised.

#### **Comments received from the Native Hawaiian community are summarized as follows:**

Cultural reviews support the acceptance of this application. No concerns were raised.

#### **Comments received from the public are summarized as follows:**

No comments were received from the public on this application.

#### **Additional reviews and permit history:**

Are there other relevant/necessary permits or environmental reviews that have or will be issued with regard to this project? (e.g. MMPA, ESA, EA)      Yes ☒      No ☐

If so, please list or explain:

- The proposed activities are in compliance with the National Environmental Policy Act.

Has Applicant been granted a permit from the State in the past?      Yes ☒      No ☐

If so, please summarize past permits:

- The applicant was granted permits DLNR/NWHI/06R006, PMNM-2007-041, and PMNM-2008-038 to conduct similar work in 2006 through 2008.

Have there been any      a) violations:      Yes ☐      No ☒  
    b) Late/incomplete post-activity reports:      Yes ☐      No ☒

Are there any other relevant concerns from previous permits?      Yes ☐      No ☒

STAFF OPINION:

DAR staff is of the opinion that Applicant has properly demonstrated valid justifications for his application and should be allowed to enter the NWHI State waters and to conduct the activities therein as specified in the application with the following special instructions and conditions, which are in addition to the Papahānaumokuākea Marine National Monument Conservation and Management Permit General Conditions. The following special conditions have been vetted through the legal counsel of the Co-Trustee agencies.

1. This permit is not to be used for nor does it authorize the sale of collected organisms. Under this permit, the authorized activities must be for noncommercial purposes not involving the use or sale of any organism, by-products, or materials collected within the Monument for obtaining patent or intellectual property rights.
2. The permittee may not convey, transfer, or distribute, in any fashion (including, but not limited to, selling, trading, giving, or loaning) any coral, live rock, or organism collected under this permit without the express written permission of the Co-Trustees.
3. To prevent introduction of disease or the unintended transport of live organisms, the permittee must comply with the disease and transport protocols attached to this permit.
4. Tenders and small vessels must be equipped with engines that meet EPA emissions requirements.
5. Refueling of tenders and all small vessels must be done at the support ships and outside the confines of lagoons or near-shore waters in the State Marine Refuge
6. No fishing is allowed in State Waters except as authorized under State law for subsistence, traditional and customary practices by Native Hawaiians.

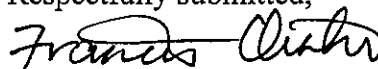
MONUMENT MANAGEMENT BOARD OPINION:

The MMB is of the opinion that the Applicant has met the findings of Presidential Proclamation 8031 and this activity may be conducted subject to completion of all compliance requirements. The MMB concurs with the special conditions recommended by DAR staff.

RECOMMENDATION:

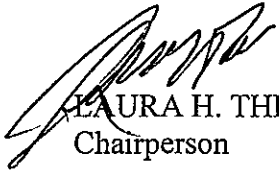
“That the Board authorize and approve, with stated conditions, a Research Permit to Dr. Ruth Gates, University of Hawaii, Hawaii Institute of Marine Biology.”

Respectfully submitted,



DAN POLHEMUS  
Administrator

APPROVED FOR SUBMITTAL

A handwritten signature in black ink, appearing to read 'Laura H. Thielen', is written over the printed name.

LAURA H. THIELEN  
Chairperson

**Papahānaumokuākea Marine National Monument**  
RESEARCH Permit Application

**NOTE:** *This Permit Application (and associated Instructions) are to propose activities to be conducted in the Papahānaumokuākea Marine National Monument. The Co-Trustees are required to determine that issuing the requested permit is compatible with the findings of Presidential Proclamation 8031. Within this Application, provide all information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Papahānaumokuākea Marine National Monument (Monument).*

**ADDITIONAL IMPORTANT INFORMATION:**

- Any or all of the information within this application may be posted to the Monument website informing the public on projects proposed to occur in the Monument.
- In addition to the permit application, the Applicant must either download the Monument Compliance Information Sheet from the Monument website OR request a hard copy from the Monument Permit Coordinator (contact information below). The Monument Compliance Information Sheet must be submitted to the Monument Permit Coordinator after initial application consultation.
- Issuance of a Monument permit is dependent upon the completion and review of the application and Compliance Information Sheet.

**INCOMPLETE APPLICATIONS WILL NOT BE CONSIDERED**

Send Permit Applications to:

Papahānaumokuākea Marine National Monument Permit Coordinator  
6600 Kalaniana'ole Hwy. # 300  
Honolulu, HI 96825  
nwhipermit@noaa.gov  
PHONE: (808) 397-2660 FAX: (808) 397-2662

**SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT REQUIRED. FOR  
ADDITIONAL SUBMITTAL INSTRUCTIONS, SEE THE LAST PAGE.**

## **Papahānaumokuākea Marine National Monument Permit Application Cover Sheet**

This Permit Application Cover Sheet is intended to provide summary information and status to the public on permit applications for activities proposed to be conducted in the Papahānaumokuākea Marine National Monument. While a permit application has been received, it has not been fully reviewed nor approved by the Monument Management Board to date. The Monument permit process also ensures that all environmental reviews are conducted prior to the issuance of a Monument permit.

### **Summary Information**

**Applicant Name:** Ruth Gates

**Affiliation:** Hawaii Institute of Marine Biology

**Permit Category:** Research

**Proposed Activity Dates:** 06/01/09-08/31/09

**Proposed Method of Entry (Vessel/Plane):** Hi'ilakai NOAA research vessel

**Proposed Locations:** Cruise Itinerary is unknown at this time and therefore this permit will cover activity for all atolls in the Monument to provide flexibility.

**Estimated number of individuals (including Applicant) to be covered under this permit:**

5

**Estimated number of days in the Monument:** 45

**Description of proposed activities:** (complete these sentences):

a.) The proposed activity would...  
identify and monitor biological indicators of coral disease and/or bleaching and assess the diversity of coral-endosymbionts in Papahānaumokuākea.

b.) To accomplish this activity we would ....  
sample coral colonies and other marine invertebrates and single-celled protists that harbor the same dinoflagellate symbionts as well as the surrounding reef waters and sediment to determine the molecular diversity of coral endosymbionts. These activities will be performed using SCUBA from small boat operations launched from the Hi'ilakai NOAA vessel.

c.) This activity would help the Monument by ...  
identifying biological indicators of disease, revealing the diversity of coral-endosymbionts in Papahānaumokuākea, and determining the affect of global climate change on the coral reef ecosystem.

**Other information or background:** The scleractinian corals provide the structural and biological framework that supports the high diversity of marine organisms that inhabit coral reef ecosystems. As such, the health status and functional integrity of coral has profound ramifications for other members of these environments (Hoegh-Guldberg 1999). Corals are susceptible to a variety of environmental disturbances that include changes in seawater temperature, salinity, UV light, pollution, and increased sedimentation (Brown 1997, Williams & Bunkley-Williams 1990, Barber et al 2001, Hoegh-Guldberg 1999). Many of these disturbances are predicted to increase in frequency and magnitude due to changes in the global climate, and these coupled with direct anthropogenic pressure has implications for even the most remote coral reef ecosystems (Hoegh-Guldberg 1999, Bellwood et al 2004). Corals respond to these environmental insults by losing their symbionts (bleaching) and/ or by exhibiting an increased susceptibility to coral diseases. These compromised biological states culminate in reduced growth and reproduction and ultimately, the death of the coral and the degradation of the habitat (Hoegh-Guldberg & Smith 1989, Jokiel & Coles 1977, Gleason & Wellington 1993, Goreau TF 1964, Kushmaro et al 1996, Glynn 1993). One of the most striking facets of corals that are exposed to deleterious conditions or exposed to disease agents is that they do not respond uniformly, that is, different species are differentially sensitive and members of the same species are not equally impacted or susceptible. At this point, our understanding of the biological factors that drive this variation in response is not well developed although we know that corals form intimate intracellular relationships with a variety of dinoflagellate symbionts, and the taxonomic specifics of these unions potentially influence the vulnerability of corals to environmental disturbance and disease causing agents. These symbionts belong to the genus *Symbiodinium*, a highly diverse group that show geographic, depth, and host specificity (LaJeunesse 2005, Stat et al 2006). Given the fundamentally important role that these symbionts play in coral biology, it is not surprising that the type of *Symbiodinium* that a coral hosts affects the growth rate and thermal tolerance of the colony (Little et al. 2004, Rowan 2004). Other biological traits that have been discussed as contributing to the vulnerability of specific corals to environmental disturbance and or disease agents include colony morphology and coral size (Loya et al 2001). Thus, a detailed understanding of morphological characteristics of the corals combined with a thorough characterization of the types of symbionts they harbor has the potential to be extremely informative about the sensitivity of the specific corals and reef assemblages to environmental shifts and disease agents.

Our previous work conducted on samples collected during September 2005 and May 2006 show that diseased *Acropora cytherea* contain a specific type of symbiotic dinoflagellate that is quite different from the type found in their healthy counterparts (Stat et al 2008). Given the implications for coral health, it is critical that we obtain an understanding of the prevalence and geographic spread of this rare symbiont in Papahānaumokuākea. The work covered by this permit request specifically addresses this need by examining the symbiont diversity harbored by corals and other marine invertebrates in the Monument and is a continuation of work initiated in 2005.



#### REFERENCES:

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- Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* espera and *Seriatopora hystrix* Dana. Journal of Experimental Marine Biology and Ecology 129: 279-304.
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- Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of Symbiodinium and scleractinian host – Symbiosis, diversity and the effect of climate change. Perspectives in Plant Ecology, Evolution and Systematics 8: 23-43.
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## **Section A - Applicant Information**

### **1. Applicant**

Name (last, first, middle initial): Gates, Ruth, D

Title: Associate Professor

**1a. Intended field Principal Investigator (See instructions for more information):**  
Michael Stat

**2. Mailing address (street/P.O. box, city, state, country, zip):**

Phone:

Fax:

Email:

For students, major professor's name, telephone and email address:

**3. Affiliation (institution/agency/organization directly related to the proposed project):**  
Hawaii Institute of Marine Biology/University of Hawaii

**4. Additional persons to be covered by permit. List all personnel roles and names (if known at time of application) here (e.g. John Doe, Research Diver; Jane Doe, Field Technician):**

Michael Stat (Field PI)

Anderson Mayfield (Field Technician)

Hollie Putnam (Field Technician)

Derek Smith (Field Technician)

## **Section B: Project Information**

### **5a. Project location(s):**

<input checked="" type="checkbox"/> Nihoa Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Necker Island (Mokumanamana)	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> French Frigate Shoals	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Gardner Pinnacles	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Maro Reef			
<input checked="" type="checkbox"/> Laysan Island	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Lisianski Island, Neva Shoal	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Pearl and Hermes Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Midway Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input checked="" type="checkbox"/> Kure Atoll	<input type="checkbox"/> Land-based	<input checked="" type="checkbox"/> Shallow water	<input type="checkbox"/> Deep water
<input type="checkbox"/> Other			

NOTE: There is a fee schedule for people visiting Midway Atoll National Wildlife Refuge via vessel and aircraft.

### **Location Description:**

The following GPS coordinates bound the region surrounding each atoll where sampling will occur. The activity will be ocean based in shallow (5 ft) to deep (80 ft) coral reef waters. The exact locations are unknown, which is dependent on coral location, presence of disease/bleaching, and the final NOAA cruise itinerary. Exact coordinates will be provided in the post cruise summary.

1	Kure Atoll	-178.19706492000	28.55825235580
1	Kure Atoll	-178.19623585400	28.29958375730
1	Kure Atoll	-178.45987884800	28.29958375730
1	Kure Atoll	-178.46070791400	28.55742328970
2	Midway Atoll	-177.19638223300	28.37419969920
2	Midway Atoll	-177.19721129900	28.13377055310
2	Midway Atoll	-177.52800864100	28.13459961920
2	Midway Atoll	-177.52800864100	28.37419969920
3	Pearl and Hermes Atoll	-176.08850981800	28.04643025580
3	Pearl and Hermes Atoll	-175.63289162600	28.04539944540
3	Pearl and Hermes Atoll	-175.63289162600	27.70729363750
3	Pearl and Hermes Atoll	-176.08954062900	27.70626282710
4	Lisianski Island	-173.67292570900	26.25150771120
4	Lisianski Island	-173.67292570900	25.83942708400
4	Lisianski Island	-174.23095155800	25.83942708400
4	Lisianski Island	-174.23095155800	26.25150771120

5	Laysan Island	-171.47900122300	25.96027179830
5	Laysan Island	-171.47725234300	25.65596666490
5	Laysan Island	-171.97918092500	25.65771554490
5	Laysan Island	-171.97918092500	25.96202067840
6	Maro Reef	-170.18133220600	25.69968866680
6	Maro Reef	-170.17958332600	25.21524888540
6	Maro Reef	-171.00505472200	25.21524888540
6	Maro Reef	-171.00505472200	25.69968866680
7	Gardner Pinnacles	-167.74832319300	25.26070709440
7	Gardner Pinnacles	-167.75087047400	24.34878019150
7	Gardner Pinnacles	-168.36221811900	24.35132747340
7	Gardner Pinnacles	-168.36476540100	25.26070709440
8	French Frigate Shoals	-165.93465851400	23.94630965900
8	French Frigate Shoals	-165.93465851400	23.56421738120
8	French Frigate Shoals	-166.45685129400	23.56421738120
8	French Frigate Shoals	-166.45685129400	23.94630965900
9	Necker Island	-164.13627752700	23.71705429230
9	Necker Island	-164.13373024500	23.20505064020
9	Necker Island	-164.92084033700	23.20505064020
9	Necker Island	-164.92338761900	23.71960157420
10	Nihoa Island	-161.66031956700	23.23816530420
10	Nihoa Island	-161.66286684900	22.94013332760
10	Nihoa Island	-162.05005369100	22.94268060940
10	Nihoa Island	-162.05260097200	23.23561802240

**5b. Check all applicable regulated activities proposed to be conducted in the Monument:**

- ☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving Monument resource
- ☐ Drilling into, dredging, or otherwise altering the submerged lands other than by anchoring a vessel; or constructing, placing, or abandoning any structure, material, or other matter on the submerged lands
- ☐ Anchoring a vessel
- ☐ Deserting a vessel aground, at anchor, or adrift
- ☐ Discharging or depositing any material or matter into the Monument
- ☒ Touching coral, living or dead
- ☐ Possessing fishing gear except when stowed and not available for immediate use during passage without interruption through the Monument
- ☐ Attracting any living Monument resource

- ☐ Sustenance fishing (Federal waters only, outside of Special Preservation Areas, Ecological Reserves and Special Management Areas)
- ☐ Subsistence fishing (State waters only)
- ☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

**6 Purpose/Need/Scope *State purpose of proposed activities:***

The purpose of this research is to identify robust biological indicators of coral disease and bleaching susceptibility. Specifically, we will characterize the diversity of symbiotic dinoflagellates (Symbiodinium) harbored by corals and examine morphological traits in healthy, diseased and bleached corals in order to identify biological traits that correlate with health state. In addition, we will evaluate the diversity of Symbiodinium in corals, other marine invertebrates and protists (Foraminifera) from Papahānaumokuākea, and free-living in the surrounding waters and sediment to examine the prevalence and geographic spread of Symbiodinium that render corals disease and/or bleach susceptible and to understand the diversity present in the Monument.

We hypothesize that:

- 1) Specific coral-Symbiodinium assemblages render corals susceptible to disease and bleaching in Papahānaumokuākea
- 2) Morphological traits in corals correlate with disease and bleaching in Papahānaumokuākea
- 3) A high diversity of Symbiodinium exist in corals, Foraminiferan, seawater and sediment surrounding the reef in Papahānaumokuākea

**7. Answer the Findings below by providing information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Monument:**

The Findings are as follows:

a. How can the activity be conducted with adequate safeguards for the cultural, natural and historic resources and ecological integrity of the Monument?

All personnel listed on this permit have attended cultural briefings (or will do so before the cruise) on the significance of Papahānaumokuākea. Our research aims to learn more about this ecosystem and provide the science to help conserve and protect it. Our impact on the environment is minimal, and does not involve the removal of whole marine invertebrate organisms. We will only take a small biopsy of each animal sampled and therefore preserve the integrity of life and the ecosystem in the monument.

b. How will the activity be conducted in a manner compatible with the management direction of this proclamation, considering the extent to which the conduct of the activity may diminish or enhance Monument cultural, natural and historic resources, qualities, and ecological integrity, any indirect, secondary, or cumulative effects of the activity, and the duration of such effects? Our sampling technique will not impact the ecological integrity of the coral reef ecosystem. The small biopsy that we remove from each colony will heal in a short period of time (weeks) and is significantly less impact than what is caused by natural predators of these organisms. We have already shown the wealth of information that can be returned from this sampling through peer reviewed publications (Stat & Gates 2007, Stat et al 2008). Our research has identified a type of endosymbiont that was likely introduced into Hawaii, and it is associated with health compromised coral. We are identifying indicators of coral disease that can be used to monitor the

health state of the ecosystem over time and make a progressive step towards understanding the coral reef ecosystem in the Monument that can aid managerial decisions towards coral reef conservation.

c. Is there a practicable alternative to conducting the activity within the Monument? If not, explain why your activities must be conducted in the Monument.

Our research aims at identifying the causes of coral reef degradation and its distribution in Papahānaumokuākea. The cause and effect of the ecosystem health can only be conducted and monitored in the location in which you want to manage. Therefore our research can only be performed in the Monument.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?

The end value which is to learn more about the diversity in the Monument, and the biological indicators driving disease/bleaching and reef degradation, far outweighs the small biopsy taken from each marine invertebrate, and the protists, water and sediment collected. Our sampling is non-lethal (except for the foraminiferans), and has a very minor effect on the organism which will recover in a short period of time (weeks).

e. Explain how the duration of the activity is no longer than necessary to achieve its stated purpose.

Our diversity study will map the patterns of coral endosymbionts in the Monument and provide a baseline for future comparisons to understand community shifts. To achieve this we need to sample at all the atolls. Monitoring of the coral endosymbiont community can then be achieved by sampling the water column and colonies showing disease/bleaching. This is an ongoing monitoring project, where the sampling is non-lethal, and will provide data to management on changes in the community structure of the ecosystem. Once the baseline diversity is determined, monitoring the environment can be achieved on a single cruise on an annual basis.

f. Provide information demonstrating that you are qualified to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

The PI and other scientists on the application are qualified coral reef biologists, recognised on an international level. They have the background and resources to conduct and complete the stated activity. There are no potential impacts from the outlined activities that we foresee.

g. Provide information demonstrating that you have adequate financial resources available to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

The science conducted in this project is performed at the Hawaii Institute of Marine Biology which houses all the facilities and infrastructure needed to perform the data analysis which is supported by funding from a partnership with the Papahānaumokuākea Monument Office.

h. Explain how your methods and procedures are appropriate to achieve the proposed activity's goals in relation to their impacts to Monument cultural, natural and historic resources, qualities, and ecological integrity.

Our research focuses on using Molecular biology and DNA analysis. To achieve this we only need small biopsies in order to get the DNA. We understand that all life is sacred in the Monument, and that coral represents the first life in Hawaiian culture. Our sampling is non-lethal and will not have an ongoing or long term impact on the environment.

i. Has your vessel has been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?  
The vessel is a federal owned NOAA ship, Hi'ialakai.

j. Demonstrate that there are no other factors that would make the issuance of a permit for the activity inappropriate.  
The applicant can not foresee any other factors that find issuance of the permit inappropriate.

#### **8. Procedures/Methods:**

A detailed sample list and the collection sites are provided in Appendix 1.

To satisfy hypothesis 1: A maximum of 30 coral colonies (5 species; 10 healthy, 20 unhealthy) will be sampled from reefs surrounding each atoll to determine if there is a correlation between disease/bleaching susceptibility and the type of Symbiodinium harbored. This number is much greater than what the actual "take" number will be, as the occurrence of bleaching and disease in all the listed species at all atolls is extremely rare. In fact, the previous 2 cruises on which we had requested this sample size, resulted in a "take" of approximately 20% of the requested total. This sample list requested thus provides flexibility and represents a perfect world scenario that will most never be encountered in nature.

To satisfy hypothesis 2: Coral colonies sampled that are unhealthy vs healthy will be photographed for further morphological analysis.

To satisfy hypothesis 3: A total of 5 colonies per marine invertebrate species and 40 Foraminifera from reefs surrounding each atoll will be sampled to determine the diversity of Symbiodinium inhabiting Papahānaumokuākea.

The samples taken from corals is a very small biopsy (<1cm). This impact is extremely minimal to the coral colony and is significantly less damaging to the coral than marine organisms that feed on coral in the area (eg. parrot fish). This sampling strategy is extremely minimal and in no way poses a threat or significant impact on the colony sampled.

Marine invertebrates and Foraminifera will be collected using SCUBA with a chisel, pliers or scissors and placed into plastic collection bags. The samples will be frozen and transported to the Hi'ialakai. The tissue from all samples will be placed into individual 1.5 ml microcentrifuge tubes containing DNA preservation buffer which destroys the integrity of the living material and poses no threat for the spread of living organisms. The remaining calcium carbonate skeleton and



tissue will be bleached to kill all living material. The samples and skeletons will be stored at the Hawaiian Institute of Marine Biology for further downstream analyses upon return to Honolulu.

All SCUBA operations will be conducted off small vessels launched from the Hi'ilakai NOAA research vessel.

**NOTE: If land or marine archeological activities are involved, contact the Monument Permit Coordinator at the address on the general application form before proceeding, as a customized application will be needed. For more information, contact the Monument office on the first page of this application.**

**9a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):**

Common name:  
see Appendix 1

Scientific name:  
see Appendix 1

# & size of specimens:  
see Appendix 1

Collection location:  
see Appendix 1

☒ Whole Organism ☒ Partial Organism

**9b. What will be done with the specimens after the project has ended?**

DNA and the remaining material will be archived at the Hawaii Institute of Marine Biology

**9c. Will the organisms be kept alive after collection?** ☐ Yes ☒ No

• General site/location for collections:

• Is it an open or closed system? ☐ Open ☐ Closed

• Is there an outfall? ☐ Yes ☐ No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

- Will organisms be released?

**10. If applicable, how will the collected samples or specimens be transported out of the Monument?**

Samples will be stored in 1.5 ml microcentrifuge tubes containing DNA preservation buffer that destroys living material. Remaining skeletons will be bleached to destroy left-over living material and stored in sealed bags. These samples will remain on board the Hi'ialakai and then transported to the Hawaii Institute of Marine Biology upon return to Honolulu.

**11. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:**

Samples that overlap with the Rappe, Toonen, and Karl lab will be shared.

**12a. List all specialized gear and materials to be used in this activity:**

SCUBA equipment  
pliers  
hammer  
chisel  
fishing collection bag to hold samples  
plastic collection bags  
DNA preservation buffer  
ethanol  
bleach  
MQ water  
razor blades  
camera and underwater housing  
laptop computer

**12b. List all Hazardous Materials you propose to take to and use within the Monument:**

bleach  
ethanol  
DNA preservation buffer (contains Guanadinium isothiocyanate, 2-mercaptoethanol)

**13. Describe any fixed installations and instrumentation proposed to be set in the Monument:**

N/A

**14. Provide a time line for sample analysis, data analysis, write-up and publication of information:**

We currently have 2 manuscripts published from our work in the Monument and have a third in review. Laboratory work, data analysis, and manuscript submission for the current project will be completed by the end of 2010.

**15. List all Applicants' publications directly related to the proposed project:**

Stat M and Gates RD. (2008) Vectored introductions of endosymbiotic dinoflagellates into Hawaii. *Biological Invasions*. 10: 579-583.

Stat M, Morris E, Gates RD. (2008) Functional diversity in coral-dinoflagellate symbiosis. *PNAS*. 105: 9256-9261.

Stat M, Pochon X, Cowie ROM, Gates RD. Specificity in communities of *Symbiodinium* in corals from Johnston Atoll. submitted.

With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury of that the information I have provided on this application form is true and correct. I agree that the Co-Trustees may post this application in its entirety on the Internet. I understand that the Co-Trustees will consider deleting all information that I have identified as "confidential" prior to posting the application.

---

Signature

Date

**SEND ONE SIGNED APPLICATION VIA MAIL TO THE MONUMENT OFFICE  
BELOW:**

Papahānaumokuākea Marine National Monument Permit Coordinator  
6600 Kalaniana'ole Hwy. # 300  
Honolulu, HI 96825  
FAX: (808) 397-2662

**DID YOU INCLUDE THESE?**

- ☐ Applicant CV/Resume/Biography
- ☐ Intended field Principal Investigator CV/Resume/Biography
- ☐ Electronic and Hard Copy of Application with Signature
- ☐ Statement of information you wish to be kept confidential
- ☐ Material Safety Data Sheets for Hazardous Materials

## Appendix 1 Gates Permit 2009

### Sampling Strategy and Collection Request for 2009

The samples taken from corals will be a very small biopsy (<1cm). This impact is minimal to the coral colony and is significantly less damaging to the coral than marine organisms that feed on coral in the area (eg. parrot fish). This sampling strategy is extremely minimal and in no way poses a threat or significant impact to the colony sampled. For other marine invertebrate hosts, a clipping of one of the tentacles will be taken which is non-lethal to the organism. Analysis of foraminiferan hosts involves taking the entire organism (which is approximately 1- 5 mm in size).

**This cruise year we will be targeting Maro and Laysan as these are the two atolls that we have not sampled from. However, as the itinerary for the research cruise has not been scheduled, a sampling strategy that provides the freedom to sample at all the atolls is included. Realistically, only a few of the atolls will be visited and so the collection number will be much less than outlined below.**

#### ***1. Collections to identify and define the types of symbiotic dinoflagellates harbored by healthy and diseased corals***

Corals belonging to the genera *Acropora* and *Pocillopora* have been chosen for this study because both are sensitive to environmental disturbance and bleach more readily than other species. In addition, data resulting from the 2005 and 2006 collections revealed that diseased *Acropora cytherea* harbor a rare and very different type of symbiont than their healthy counterparts at French Frigate Shoals (Stat et al. 2008). *Porites lobata* and *Montipora capitata* have also been chosen for this study because they are common and are widely distributed within the Hawaiian archipelago and throughout the Pacific, they exhibit differences in their susceptibility to bleaching and disease, and both species are being developed as models for work focusing on functional aspects of the symbiosis being conducted at HIMB. In addition, ongoing sampling for this objective is important for understanding the changes in the symbiont community that occur over time as a result of increases in coral bleaching and disease.

We are requesting a permit to cover the collection of thirty corals (a statistically relevant number) representing each species and health state, for all ten atolls. In reality, the number of samples collected at each location in Papahānaumokuākea will reflect the incidence of disease and/or bleaching, and our past experiences suggest that individuals representing the compromised health states are rarely encountered. For example, on the September 2005 expedition, coral disease was limited to a single host species, *A. cytherea*, found at one reef location, French Frigate Shoals. As such, we have designed a sampling strategy that provides the researchers the flexibility to take advantage of chance encounters with diseased and/or bleached individuals of the target species at each site visited but that translates as a substantial overestimation of the actual number of corals that will be collected. In addition, the cruise schedule of the Hi'ialakai has not been determined and therefore we are listing all ten atolls

within the monument for this objective, however it is very unlikely that we will visit all of the atolls.

**Coral Species (n = 10 for healthy, n = 20 for unhealthy at each atoll)**

**Nihoa**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

**Necker**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

**French Frigate Shoals**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

**Gardner**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

**Maro**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

**Laysan**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

#### **Lisianski**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

#### **Pearl and Hermes**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

#### **Midway**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

#### **Kure**

<b>Coral Species</b>	<b>Common Name</b>	<b>Sample Number</b>
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

**Total number request of coral samples for disease study: 1230 biopsies**

**Estimate of actual collection: < 250 coral biopsies**

**Note that the majority of samples (67%) will be collected from diseased and bleached coral and therefore will not affect healthy coral colonies. Corals species sampled collection 1 (described above) and collection 2 (described below) that overlap represent a single sample.**

## **2. Collections to define the diversity and distribution of symbiotic dinoflagellates across Papahānaumokuākea**

This study focuses on the types of symbiotic dinoflagellates found in a diversity of corals and other marine invertebrates and foraminifera from locations that cross the archipelago (see below) and is a continuation of work initiated in 2005. To date we have a portion of the total coral and foraminiferan sample request collected from all atolls except Maro and Laysan. We are also requesting an increase to the number of forams to be sampled from 20 to 40 forams per species per atoll. This is due to the extremely high abundance observed on the 2008 cruise (several hundred per square foot in some areas).

**The sample number for a host species that has been reached on previous cruises will not be re-sampled in this upcoming cruise.**

### **1. Marine Invertebrates:**

**Total is 5 colonies per species per atoll (27 species X 5 colonies X 10 atolls)**

<b>Coral Species</b>	<b>Common Name</b>
<i>Acropora humilis</i>	Finger Staghorn Coral
<i>Acropora cytherea</i>	Table Coral
<i>Acropora nasuta</i>	Branching staghorn coral
<i>Pocillopora damicornis</i>	Lace Coral
<i>Pocillopora meandrina</i>	Cauliflower Coral
<i>Pocillopora eydouxi</i>	Antler Coral
<i>Pocillopora ligulata</i>	Cauliflower Coral
<i>Porites brighami</i>	Brigham's Coral
<i>Porites lichen</i>	Lichen Coral
<i>Porites lobata</i>	Lobe Coral
<i>Porites duerdeni</i>	Thick Finger Coral
<i>Porites solida</i>	Solid Coral
<i>Montipora capitata</i>	Rice Coral
<i>Montipora patula</i>	Sandpaper Rice Coral
<i>Montipora flabellata</i>	Blue Rice Coral
<i>Leptastrea bewickensis</i>	Bewick Coral
<i>Pavona varians</i>	Corrugated Coral
<i>Pavona duerdeni</i>	Porkchop Coral
<i>Fungia scutaria</i>	Oval Mushroom Coral
<i>Gardineroseris planulata</i>	Honeycomb coral
<i>Cyphastrea ocellina</i>	Ocellated Coral
<i>Leptoseris mycetoseroides</i>	Ridge Coral
<i>Psammocora niertrazi</i>	Nierstrasz' Coral
<b>Other Invertebrate hosts</b>	
<i>Aiptasia pulchella</i>	Glass, Tube, Rock Anemone
<i>Palythoa caesia</i>	Pillow Zoanthid
<i>Sarcothelia edmondsoni</i>	Blue Soft Coral



*Cassiopea sp.*

Upside-Down Jellyfish

**Total number request of marine invertebrate samples for diversity study: 1350**

**Number collected to date: 317**

**Number remaining to collect: 1033**

**Estimate of actual final collection: 500 (This is due to most of the species listed above found at only some of the atolls, but gives the freedom for opportunistic sampling)**

## **2. Foraminifera**

**Total is 40 forams per species per atoll (3 species X 20 colonies X 10 atolls)**

*Amphisorus hemprichii*

*Marginopora kudakajimaensis*

*Sorites sp*

**Total number request of foram samples for diversity study: 1200**

**Number collected to date: 240**

**Estimate of actual final collection: 800**

## Papahānaumokuākea Marine National Monument Compliance Information Sheet

**1. Updated list of personnel to be covered by permit. List all personnel names and their roles here (e.g. John Doe, Diver; Jane Doe, Field Technician, Jerry Doe, Medical Assistant):**

Michael Stat, Field PI; Rob Toonen, diver (has own permit application); Matt Iachhei, diver; John Puritz, diver; Derek Smith (has own permit application), diver.

**2. Specific Site Location(s): (Attach copies of specific collection locations):**

The following GPS coordinates bound the region surrounding each atoll where sampling will occur. The activity will be ocean based in shallow (5 ft) to deep (80 ft) coral reef waters. The exact locations are unknown, which is dependent on coral location, presence of disease/bleaching, and the final NOAA cruise itinerary. Exact coordinates will be provided in the post cruise summary.

1	Kure Atoll	-178.19706492000	28.55825235580
1	Kure Atoll	-178.19623585400	28.29958375730
1	Kure Atoll	-178.45987884800	28.29958375730
1	Kure Atoll	-178.46070791400	28.55742328970
2	Midway Atoll	-177.19638223300	28.37419969920
2	Midway Atoll	-177.19721129900	28.13377055310
2	Midway Atoll	-177.52800864100	28.13459961920
2	Midway Atoll	-177.52800864100	28.37419969920
3	Pearl and Hermes Atoll	-176.08850981800	28.04643025580
3	Pearl and Hermes Atoll	-175.63289162600	28.04539944540
3	Pearl and Hermes Atoll	-175.63289162600	27.70729363750
3	Pearl and Hermes Atoll	-176.08954062900	27.70626282710
4	Lisianski Island	-173.67292570900	26.25150771120
4	Lisianski Island	-173.67292570900	25.83942708400
4	Lisianski Island	-174.23095155800	25.83942708400
4	Lisianski Island	-174.23095155800	26.25150771120
5	Laysan Island	-171.47900122300	25.96027179830
5	Laysan Island	-171.47725234300	25.65596666490
5	Laysan Island	-171.97918092500	25.65771554490
5	Laysan Island	-171.97918092500	25.96202067840
6	Maro Reef	-170.18133220600	25.69968866680
6	Maro Reef	-170.17958332600	25.21524888540

6	Maro Reef	-171.00505472200	25.21524888540
6	Maro Reef	-171.00505472200	25.69968866680
7	Gardner Pinnacles	-167.74832319300	25.26070709440
7	Gardner Pinnacles	-167.75087047400	24.34878019150
7	Gardner Pinnacles	-168.36221811900	24.35132747340
7	Gardner Pinnacles	-168.36476540100	25.26070709440
8	French Frigate Shoals	-165.93465851400	23.94630965900
8	French Frigate Shoals	-165.93465851400	23.56421738120
8	French Frigate Shoals	-166.45685129400	23.56421738120
8	French Frigate Shoals	-166.45685129400	23.94630965900
9	Necker Island	-164.13627752700	23.71705429230
9	Necker Island	-164.13373024500	23.20505064020
9	Necker Island	-164.92084033700	23.20505064020
9	Necker Island	-164.92338761900	23.71960157420
10	Nihoa Island	-161.66031956700	23.23816530420
10	Nihoa Island	-161.66286684900	22.94013332760
10	Nihoa Island	-162.05005369100	22.94268060940
10	Nihoa Island	-162.05260097200	23.23561802240

**3. Other permits (list and attach documentation of all other related Federal or State permits):**

State of Hawaii, Department of Land & Natural Resource, Division of Aquatic resources, Special Activity Permit No. 2008-99.

**3a. For each of the permits listed, identify any permit violations or any permit that was suspended, amended, modified or revoked for cause. Explain the circumstances surrounding the violation or permit suspension, amendment, modification or revocation.**

N/A

**4. Funding sources (Attach copies of your budget, specific to proposed activities under this permit and include funding sources. See instructions for more information):**

This project is supported by the National Marine Sanctuary program and Hawaii Institute of Marine Biology Reserve Partnership, NMSP MOA 2005-008/66882.

**5. Time frame:**

Activity start: 2005

Activity completion: ongoing

Dates actively inside the Monument:

From: 06/08/09

To: 07/04/09

Describe any limiting factors in declaring specific dates of the proposed activity at the time of application:

Personnel schedule in the Monument:

Field PI Michael Stat will be in the monument for the duration of the cruise and will collect samples as outlined in the Gates Permit application in the region boundaries outlined above. Specific locations for collections will be submitted in the post cruise report. Other listed personnel are to assist the Field PI Michael Stat in the collections. Rob Toonen and Derek Smith will be on the same cruise and have their own permit applications. Either John Puritz or Matt Iacchei will be going on the cruise for the entire duration to support Michael Stat.

**6. Indicate (with attached documentation) what insurance policies, bonding coverage, and/or financial resources are in place to pay for or reimburse the Monument trustees for the necessary search and rescue, evacuation, and/or removal of any or all persons covered by the permit from the Monument:**

All personnel will be covered by University of Hawaii and DAN divers insurance.

**7. Check the appropriate box to indicate how personnel will enter the Monument:**

- ☒ Vessel  
☐ Aircraft

Provide Vessel and Aircraft information: NOAA vessel Hi'ialakai is a federal research vessel.

**8. The certifications/inspections (below) must be completed prior to departure for vessels (and associated tenders) entering the Monument. Fill in scheduled date (attach documentation):**

- ☐ Rodent free, Date:  
☐ Tender vessel, Date:  
☐ Ballast water, Date:  
☐ Gear/equipment, Date:  
☐ Hull inspection, Date:

**9. Vessel information (NOTE: if you are traveling aboard a National Oceanic and Atmospheric Administration vessel, skip this question):**

Vessel name:

Vessel owner:  
Captain's name:  
IMO#:  
Vessel ID#:  
Flag:  
Vessel type:  
Call sign:  
Embarkation port:  
Last port vessel will have been at prior to this embarkation:  
Length:  
Gross tonnage:  
Total ballast water capacity volume (m3):  
Total number of ballast water tanks on ship:  
Total fuel capacity:  
Total number of fuel tanks on ship:  
Marine Sanitation Device:  
Type:

Explain in detail how you will comply with the regulations regarding discharge in the Monument. Describe in detail. If applicable, attach schematics of the vessel's discharge and treatment systems:

Other fuel/hazardous materials to be carried on board and amounts:

Bleach 2 Liters

Ethanol 1 Liter

DNA preservation buffer (contains Guanadinium isothiocyanate, 2-mercaptoethanol) 25 mL

Provide proof of a National Oceanic and Atmospheric Administration (NOAA) Office of Law Enforcement-approved Vessel Monitoring System (VMS). Provide the name and contact information of the contractor responsible for installing the VMS system. Also describe VMS unit name and type:

VMS Email:

Inmarsat ID#:

#### **10. Tender information:**

On what workboats (tenders) will personnel, gear and materials be transported within the Monument? List the number of tenders/skiffs aboard and specific types of motors:

#### **Additional Information for Land Based Operations**

**11. Proposed movement of personnel, gear, materials, and, if applicable, samples:**

**12. Room and board requirements on island:**

**13. Work space needs:**

**DID YOU INCLUDE THESE?**

- ☐ Map(s) or GPS point(s) of Project Location(s), if applicable
- ☐ Funding Proposal(s)
- ☐ Funding and Award Documentation, if already received
- ☐ Documentation of Insurance, if already received
- ☐ Documentation of Inspections
- ☐ Documentation of all required Federal and State Permits or applications for permits